Welcome to BIOC 318, Laboratory Studies in Applied Microbiology

NOTE: This is the most recent syllabus for the course, used in fall 2019. The course structure will be mostly the same for spring 2020. Dates will be changed and some reorganization will be necessary to accommodate the spring calendar.

Many studies of samples taken from a wide variety of sources have revealed that between 0.01 and 0.1% of all bacterial cells that can be seen in a light microscope are "uncultivable" in the laboratory.[1] This means that for every bacterial species successfully isolated from an environmental sample, as many as 10,000 species remain undetected. Scientists have estimated that up to one trillion microbial species exist on Earth, about 0.001% of which are actually known.[2] If the one trillion estimate is accurate, then if 100 Rice students each discover 100 new species every year (obviously not a realistic expectation), it will take 100 million years to discover all of them.

Why should these findings interest us? It means that the opportunities for discovery are wide open. If you can overcome the significant challenges that accompany the search for new bacterial species you may indeed go where no one else has gone. This semester we will focus our efforts on finding "wild" bacteria of a particularly important genus.

Our project

Example of some of the work you will conduct in this course. (A) Agar plates with bacterial unknowns, 72h following aerobic incubation at 30˚C. *(B)* Identically streaked agar plates incubated anaerobically for 72h, revealing relationship to oxygen.

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[1] D'Onofrio, Anthony, Jason M. Crawford, Eric J. Stewart, Kathrin Witt, Ekaterina Gavrish, Slava Epstein, Jon Clardy, and Kim Lewis . "Siderophores from Neighboring Organisms Promote the Growth of Uncultured Bacteria." Chemistry & Biology (2010): 254-64.

[2] [2]Offord, Catherine. "Earth: Home to 1 Trillion Microbial Species." The Scientist (2016): http://www.the-scientist.com/?articles.view/articleNo/46016/title/Earth--Home-to-1-Trillion-Microbial-Species/

Competitive and even cooperative social interactions among bacterial strains define much of the ecology of soil. As a class we will attempt to isolate and identify "wild" species of *Streptomyces* bacteria, in support of a project by the Shamoo lab to study the evolution of such interactions. By learning how these mechanisms came to be, the Shamoo lab also seeks to learn ways of manipulating bacteria to make them do things that they do not normally do. *Streptomyces* are the source of some 75% of medically important antibiotics. One potential side benefit, of no small consequence, may be to induce these organisms to produce novel antibiotics.

Project work to date has revealed that published methods for isolating *Streptomyces* colonies may not be the best approach to isolating species from our samples. This semester we will systematically explore alternative methods that have, so far, shown promise.

To broaden our objectives, we will also study the diversity of bacteria in our samples, with each team focusing on a particular subset of known taxa. For this study and to confirm the identities of suspected *Streptomyces* isolates, you will prepare samples for 16S rRNA sequencing followed by a BLAST search for a match to known species.

The accuracy of the 16S rRNA sequencing method for bacterial identification is only as good as its database. Therefore you will employ a second approach to bacterial identification, namely characterization by phenotype. This project will require mastery of culture methods, staining methods, proficiency with the light microscope, and careful, systematic use of assays and observations leading to confirmation of a species' identity. There is even a possibility, however remote, that you will discover a previously unknown bacterial species or strain of *Streptomcyes*. Successful completion of this work will require that you work effectively with your team, keep meticulous records, produce the best quality work, and write up your findings toward the end of the course.

Objectives and learning outcomes

The main goal of this course, as with any experiential course, is personal development. We want you to build on skills that you've already acquired and to learn new ones as well. Below we summarize our most important learning objectives and outcomes.

1. Learn to work as a productive member of a research team on a long term project
2. Be able to access and employ published reference materials, current primary literature, and other information sources in order to advance your understanding of the properties of bacteria and how to isolate and characterize them
3. Learn to integrate your previous and newly acquired experience into development of new techniques and strategies
4. Improve your powers of observation, such as the ability to use physical and metabolic differences to distinguish strains and species of bacteria and to draw information from microscopic observations
5. Learn to make the decisions that are necessary in order to conduct a systematic open-ended investigation

6. Learn to make your work accessible to yourself and others by documenting your activities and findings in a well-organized laboratory notebook, by contributing to our laboratory's database, and by summarizing your work in a clearly written report
7. Learn and practice good laboratory "citizenship," including respect for people, the facility, and equipment
8. Acquire proficiency in essential methodologies and use your newly acquired skills to advance our project
9. Learn to appreciate the value and potential of pure science, that is, the pursuit of knowledge for its own sake

Please see the Syllabus for course details, course timetable, and a list of assignments with due dates.

Syllabus

The information contained in this syllabus and related pages may be subject to change, with reasonable notice, at the discretion of the instructor.

Instructor and office hours

David Caprette, Teaching Professor, caprette@rice.edu, 713-348-3498 (campus extension 3498), office located in HBH 08B (Herman Brown Hall basement)

Note that this is a temporary location during renovations of the Anderson Biological Laboratory Building

Office hours are variable (see HERE for a current schedule)

Preparing for the course

Please plan to apply the skills and best practices that you learned in BIOC 211, Intermediate Experimental Biosciences (course prerequisite) and in BIOC 311, Advanced Experimental Biosciences, if you have had the opportunity to complete that course. Please check the Course Schedule (below), review the meeting schedule for your lab section, and block out the scheduled times on your calendar. You must not schedule events that conflict with any of the listed in-lab activities, nor let anyone schedule a conflicting event for you.

Required materials

You will need eye protection for this course, consisting of a pair of lab goggles or laboratory safety glasses with side protection. A lab coat will be provided. There is no textbook but you will need to obtain a laboratory notebook. You are welcome to start with a partly filled notebook from another course, even one obtained from another student. New notebooks can be purchased from the campus store or ordered directly from the distributor. Please purchase a Hayden-McNeil Carbonless TOP Permanent Lab Notebook, 50 Set or 100 Set from Amazon, from WH Freeman, or the Campus store (ISBNs below).

Chemistry Top Bound 100 pages, ISBN 978-1-930882-00-3
or
Chemistry Top Bound 50 pages, ISBN 978-1-930882-50-8

Course timetable

All meetings begin at 1 pm unless otherwise noted. All meetings will be held in ABL 127. Documents to help you prepare for lab (listed under the heading "Preparation" below) can be found in Files and are also accessible from the side menu on the BIOC 318 web pages. Documents that will be provided in class as handouts are marked with an asterisk*.

To print a file, download it first then print as usual. Attempting to print directly from a Canvas window may not work.

<table>
<tr><td colspan="4">First 2 1/2 weeks

Obtain and purify bacterial isolates, genomic DNA, and PCR product for 16S rRNA analysis</td></tr>
<tr><th>Sec 001</th><th>Sec 002</th><th>Preparation</th><th>Description of activities</th></tr>
<tr><td>Weds Oct 16, 1:00 pm</td><td>Thur Oct 17, 1:00 pm</td><td>Review the course home page in Canvas and this syllabus and have questions ready
In the "Lab documents" folder in Files, review the documents "General lab safety," "BIOC 318 safety," "Aseptic technique," "Making serial dilutions," Preparing spread plates," and "Isolating Streptomyces;" be prepared for a quiz on the material
Look over the Day 1 outline in the Daily outlines folder in Files
Peruse the documents "Preparing R2A agar plates," "Using the autoclave," "Isolating colonies," and "Colony</td><td>Introductory classroom meeting
Prepare agar media and pour plates to be used for isolating colonies
Short quiz and lab tour
Prepare samples for isolating Streptomyces from soil and/or water suspensions
Dilute and spread samples onto agar media and incubate
Practice sessions on isolating colonies and making streak plates</td></tr>
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		morphology;" you won't be quizzed on this material	
Mon Oct 21	Tues Oct 22	• Review the PowerPoint slides in "Course introduction," posted in Files • Look over the Day 2 outline • Review the documents "Gram stain" and "Viewing Gram stains*" and be prepared for a short quiz	• Identify unique colonies and streak isolated colonies onto agar plates (purification step 1) • Learn the Gram stain method • Learn and practice microscopic observation of Gram stains • Check streak plates the <u>next</u> day and re-streak as described in the Day 2 outline (purification step 2) <u>if there is sufficient growth</u>
Weds Oct 23	Thurs Oct 24	• Review the documents "Relationship to oxygen," and "Agar slant tubes" and be prepared for a short quiz • Look over the Day 3 outline and the document (you will not be quizzed on this material)	• Prepare agar slant tubes for storing isolates and broth tubes for liquid cultures • Re-streak plates if there is sufficient growth (purification step 3) • Set up assays for oxygen requirements

Mon Oct 28	Tues Oct 29	• Again review the documents "Relationship to oxygen" and "Agar slant tubes"	• Check your isolates for contamination; for 3x purified isolates, inoculate and incubate slant tubes and broth tubes • Remove plates from anaerobic jars and assess cultures for oxygen requirements
Weds Oct 30	Thurs Oct 31	• Review the document "Sequencing" (you will not be quizzed on this material) • The document "BASIC PROTOCOL 1" describes how to prepare liquid cultures for DNA extraction	• Conduct genomic DNA isolation and PCR on the DNA • Continue to work with slower-growing samples • Assemble notebook copy pages, turn in for mid-course evaluation

Remainder of course **Select and BLAST 16S rRNA sequences** **Characterize isolates by phenotype** **Complete the identifications and report findings**			
Mon Nov 4	Tues Nov 5	• Review the document BLAST • Download 16S rRNA sequences and chromatograms and necessary software	• Analyze chromatograms, select and BLAST DNA sequences • Re-streak cultures as needed to obtain material for phenotype characterization
Weds Nov 6	Thurs Nov 7	• Review the document "Viewing living bacteria" • Review the document "Using Bergey's manuals"	• Learn and practice microscopic examination of living bacteria • Learn to conduct catalase and oxidase assays • Each team signs out a Bergey's manual • Begin to assign isolates to Bergey's "Groups" • Individual write-ups will be due Wednesday Nov 27 (submitted on line)

Mon Nov 12 through Weds Dec 4	Tues Nov 13 thru Thurs Dec 5	• Review the document "Identify_Actinobacteria" • Attempt to identify your isolates based upon phenotype, compare with BLAST results	• Conduct other assays and observations as needed • Attempt to characterize probable *Streptomyces* species • Review, assemble, and submit notebook copy pages by noon the last day of classes • Complete and submit the team write-up by noon the last day of finals

Course grade

Your grade will be based upon your preparation and lab performance (30%), laboratory notebook (30%), an individual write-up (30%) and a team-written paper (10%).

Additional information and policies

Absences. Because the work in this course is part of a long term goal-oriented project, the concept of a "make-up lab" does not apply. Missing an important event is a lost opportunity that cannot be made up. As far as your course grade is concerned, absences from required meetings will be excused for the usual reasons, e.g., illness or family emergency. Absences due to circumstances within a student's control (e.g., forgetting to show up or travel for interviews) cannot be excused and will be reflected in the performance grade.

Lab safety. All participants must engage in safe laboratory practices and abide by specific "rules" set down for this course.

Late assignments. An assignment that is submitted late will lose 10% per day including holidays and weekends.

Honor code policy. As with all biosciences courses, the Rice University Honor Code applies to all personal conduct and assignments. All work submitted under your name must be your own work, not copied from another source nor completed with unauthorized assistance. Compliance with the honor code includes providing accurate information when requested, such as honest evaluations of yourself and your teammates.

Students with disabilities. Any student with a disability needing academic adjustments or accommodations should contact Dr. Caprette as soon as possible and also contact Disabled Student Services in the Ley Student Center.